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Vaccine-induced T cells against Sars-Cov-2 and its Omicron variant in B cell-depleted lymphoma patients after CART

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Abstract:

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Letter to Blood

Vaccine-induced T cell responses against Sars-Cov-2 and its Omicron variant in B cell-depleted lymphoma patients after CART therapy

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TO THE EDITOR:

COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) containing the spike (S) and nucleocapsid (N) proteins[1,2]. The S protein consists of the S1/S2 components and the virus enters cells through binding of the receptor-binding domain (RBD) within the S1 protein[3] to the angiotensin-converting enzyme-2 (ACE-2) receptor[1,4]. Unfortunately, patients with hematologic malignancies evidence dramatically increased mortality rates following SARS-CoV-2 infection[5,6] and patients infected following chimeric antigen receptor T cell (CART) cell treatment for B cell malignancies experience mortality rates as high as 33%[7].

The development of anti-SARS-CoV-2 antibodies following an active infection and/or vaccination, is crucial for limiting disease severity, protection from future COVID-19 infections, and controlling viral transmission[8,9]. Unfortunately, patients with the most common hematologic malignancies, namely B cell lymphomas, develop insufficient antibody responses to mRNA vaccines, by virtue of treatment-induced immunosuppression[10,11]. However, in addition to antibody responses, development of antiviral T cells has been shown to improve survival in patients with COVID-19 and hematologic cancers[12], thus vaccine-induced T cell responses have the potential to “salvage” protective immunity in patients with B cell lymphoma. This may be particularly important in patients receiving one of the most effective B cell-depleting treatments, CD19 CAR T cells. In this study we performed a prospective, comprehensive monitoring of vaccine-induced anti-SARS-CoV-2 antibody and T cell immunity in B cell lymphoma patients treated with CART.

In this prospective, single-center clinical study evaluating immune responses to COVID-19 mRNA vaccines authorized by the US Food and Drug Administration, we enrolled patients with B cell lymphomas treated with CD19 CAR T cells (N=18) and healthy controls (N=10) (Supplemental Table 1). Most patients had large B cell lymphomas, followed by follicular

lymphoma, mantle cell lymphoma, and primary-mediastinal large-B cell lymphoma. The vast majority of patients had advanced-stage disease and were heavily pretreated. Ten patients received the mRNA-1273 (Moderna) vaccine, and 8 received the BNT162b2 (Pfizer/BioNTech) vaccine. All but two patients were in complete remission at the time of vaccination (Supplemental Figure 1). Two patients received the vaccine before CART, 13 patients received the vaccine after CART (median 5 weeks; range 3-64), and 3 patients were treated with CAR T cell therapy followed by allogeneic stem cell transplantation (alloSCT) and then received the vaccine (median 71 weeks; range 67-76).

When we analyzed 14 of our 18 CART patients who had samples for either the pre-vaccine or the post 2nd dose timepoints for IgG antibodies against different SARS-CoV-2 proteins, we found that after the two initial doses of COVID-19 mRNA vaccine, none of the 13 B cell lymphoma patients developed an immune response against the RBD, S1, S2, or N proteins. In contrast, all healthy controls (HC) showed a highly significant vaccine-induced increase in antibody titers against all vaccine-generated viral proteins; as expected, none developed anti-N protein antibodies, as the N protein is not part of the vaccine formulation, and N protein antibodies discriminate vaccinated patients from SARS-CoV-2-infected patients (Figure 1A, Supplemental Figure 2C). Accordingly, sera from B cell lymphoma patients without prior CART treatment and vaccinated HC showed an increase from baseline in anti-RBD IgG antibody titers after 2nd and 3rd doses, while CART-treated patients did not demonstrate detectable antibodies (Figure 1B). Consistent with this, CART patients did not evidence neutralizing activity at any of the three post-vaccine timepoints, while controls demonstrated almost 100% inhibition of viral entry after 2-3 doses of vaccine (Figure 1C). Importantly, although control subjects' antiviral antibodies showed reduced reactivity toward the Omicron variant as compared to the original SARS-CoV-2 virus (Supplemental Figure 2D), three doses of mRNA vaccine yielded some degree of Omicron-variant neutralization (Figure 1C). In contrast, among CART patients,

polyclonal sera showed no measurable inhibition of the currently prevalent Omicron variant (Figure 1C). We asked whether the lack of vaccine-induced anti-SARS-CoV-2 immune responses was due to nonspecific, global, treatment/disease-induced immunosuppression; we found that while CART patients did, indeed, have lower levels of total IgG, IgM, and IgA (Supplemental Figure 2A), these patients actually had maintained normal levels of IgG antibodies against recall antigens, such as Influenza A, tetanus toxoid, Epstein–Barr virus, and herpes simplex (Supplemental Figure 2B). Looking to explain the specific absence of any de-novo humoral immunoreactivity, we confirmed associated depletion of peripheral-blood B cells in CART patients compared with intact B-cell compartments in all other subjects, including HCs, heavily pretreated non-CART treated lymphoma patients, and patients with active COVID-19 (Figures 1D and 1E). Impressively, CD19-CART treatment selectively depleted all $CD19^+/CD20^+$ B cells from our patients' blood, thus eradicating the immune-cell compartment secreting anti-SARS-CoV-2 antibodies (Supplemental Figure 2E), yet left $CD19^-/CD38^+$, long-lived memory plasma cells intact. Indeed, at baseline, CART patients showed even higher numbers of plasma cells than the healthy vaccinated controls (Figure 1E). Combined, our serological findings indicate that while CART patients are able to generate memory antibody responses, e.g., against antigens encountered prior to B cell-depleting CART therapy, they are incapable of mounting de-novo antibody responses against novel antigens, such as SARS-CoV-2.

We next asked whether CART patients evidenced anti-SARS-CoV-2 T cell generation despite the absence of humoral antiviral immunity. As a first step, we evaluated the general T cell immunoreactivity of our CART patients and found that T cell responses to T cell receptor (TCR) crosslinking or a mixture of recall antigens was comparable to our HCs (Supplemental Figure 3 A+B). Even more importantly, we found that, indeed, CART patients showed vaccine-induced $CD4^+$ and $CD8^+$ -specific T cells in the blood, targeting the S protein of SARS-CoV-2 (Figure 2 A). After two initial doses of mRNA vaccine, numbers of virus-specific T cells were equal to and

sometimes even surpassed those of healthy, vaccinated controls as well as patients with active COVID-19 infection (Figure 2A). The antiviral $CD8^+$ T cells showed strong cytotoxic potential upon stimulation with the SARS-CoV-2 S peptides as indicated by coexpression of granzyme B, perforin, and CD107a, a marker of recent degranulation (Supplemental Figure 4). Over the course of three doses of the mRNA COVID-19 vaccine, our CART patients showed an increase in S protein-specific $CD4^+$ and $CD8^+$ T cells, comparable to those seen in lymphoma patients without prior CART treatment as well as in HCs (Figure 2B). Most of the immunodominant epitopes of the anti-SARS-CoV-2 $CD4^+$ T cells were within the S1 component of the S fusion protein, whereas for SARS-CoV-2-specific $CD8^+$ T cells, most of the immunodominant epitopes presumably were present at the C-terminal end of the S2 component (Figure 2C). Importantly, while a decrease in T cell reactivity was observed when exposed to the S protein Omicron variant, most of the polyclonal vaccine-induced $CD4^+$ and $CD8^+$ T cells also recognized this immune-escape variant of ancestral SARS-CoV-2 virus (Supplemental Figure 5).

Limitations of this study include a relatively small sample size and a somewhat limited functional assessment of vaccine-induced T cell responses. However, we have been able to show that B cell lymphoma patients treated with CD19-CART are rendered incapable of developing vaccine-induced antibody responses against novel antigens such as SARS-CoV-2 as a result of potent B cell depletion. We also demonstrate that these same patients are still capable of developing anti-SARS-CoV-2 T cells that even recognize the Omicron “immune-escape”[13] variant. Ongoing studies are investigating whether these Omicron-reactive T cells are indeed protective, especially in light of a certain degree of lymphopenia typically present in our patient population (Supplemental Figure 6), as well as whether additional measures, such as reimmunization with previous vs. heterologous vaccine[14] can effectively seroconvert B cell lymphoma patients after CD19-CART treatment.

DECLARATIONS

Competing interests

S.D. serves on advisory boards for Bristol-Myers Squibb, Incyte, and Atara Biotherapeutics. The remaining authors declare that they do not have any competing interests.

Authors' contributions

D.A. designed the study, performed experiments, analyzed the data, made figures, and wrote the manuscript. T.L., N.M.H., K.K.S., J.S.H., S.V.N., P.M., S.T.L., J.Y.L., E.V.M., K.H., J.B., M.K., J.A.Y., A.P.R., and S.D. analyzed data and wrote the manuscript. D.O., T.I., and S.V.A. processed patient samples and performed experiments. F.L. and H.D.M. collected patient data and wrote the manuscript. X.F. performed experiments and wrote the manuscript. S.B. and P.L. collected patient material.

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FIGURE LEGENDS

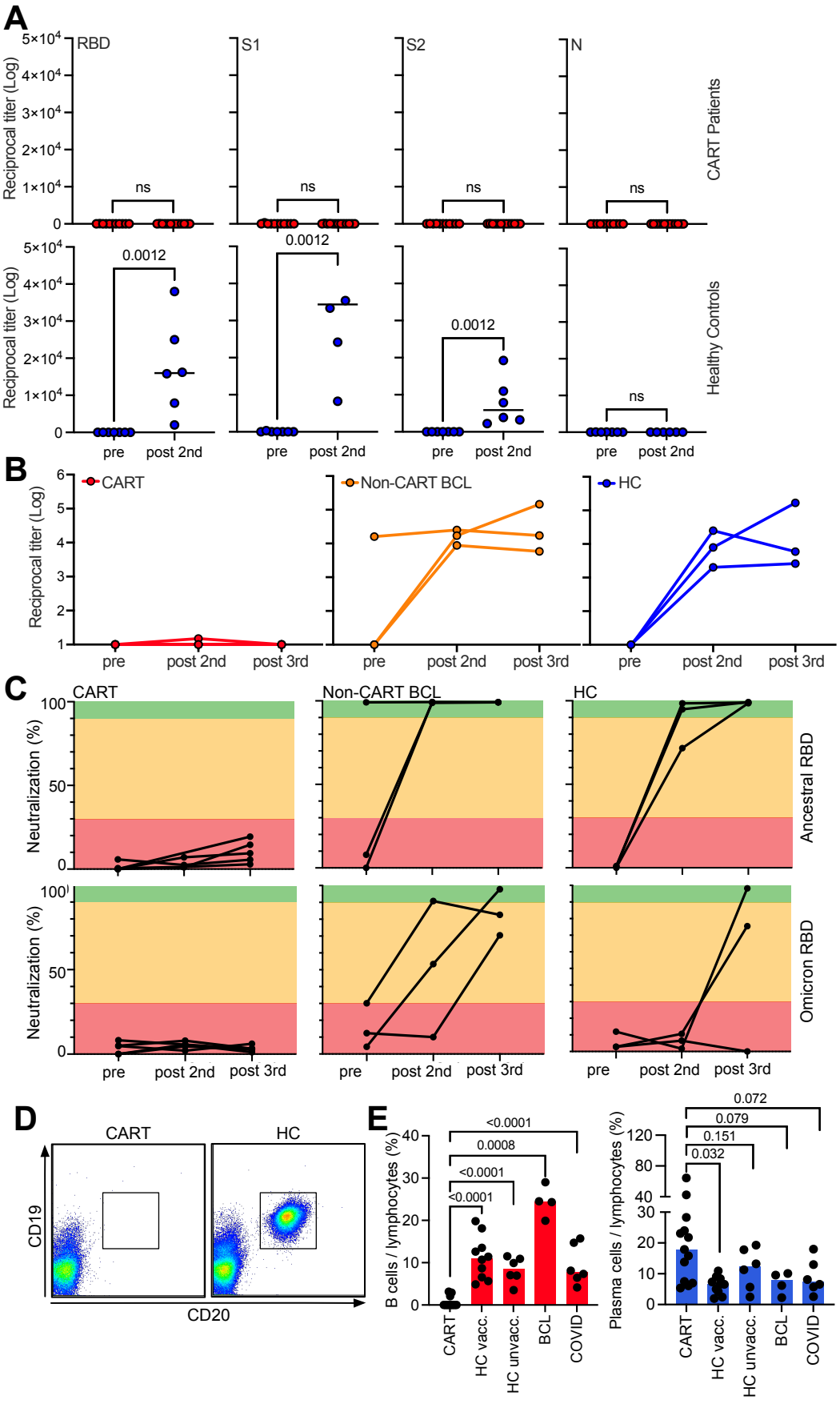
Figure 1: Anti-SARS-CoV-2 B cell responses in CART patients after 2-3 doses of a COVID-19 mRNA vaccine

(A) Titers of IgG antibodies against different full-length recombinant SARS-CoV-2 proteins were measured in CART patients (upper panel in red) and healthy controls (HC; lower panel in blue) before (11 CART patients, 7 HC) and after (13 CART patients, 6 HC) two doses of a COVID-19 mRNA vaccine. **(B)** For 5 CART patients (red), 3 B cell lymphoma (BCL) patients without prior CART treatment (yellow), and 3 HC (blue) samples were available for all three timepoints and anti-RBD titers were measured pre-vaccine, after the second dose and after the third dose. **(C)** Neutralizing activity pre-vaccine, after the second dose and after the third dose of a COVID-19 mRNA vaccine in the peripheral blood of the same groups. Green, orange, and red areas indicate different degrees of inhibition (green: >90%, orange: 30-89%, red: <30%). Neutralizing activity is shown for both the original “ancestral” SARS-CoV-2 RBD protein (upper panel) and for its omicron variant (lower panel). **(D)** Example of a flow cytometric analysis of B cell subpopulations in the peripheral blood of a CART patient (2123-038) and a HC (2123019 -right), respectively, prior to the first dose of the vaccine. Dot plots show CD19+/CD20+ B cells after gating on CD3-/CD56-/CD14- lymphocytes. **(E)** Percentages of peripheral blood CD19+/CD20+ B cells (red bars) and CD19-/CD38+ were determined in 13 CART patients, 10 vaccinated HC, 4 B cell lymphoma patients without prior CART at the pre-vaccine timepoint as well as in 6 unvaccinated HC and 7 patients with active COVID-19. Bars indicate medians. Differences between groups were analyzed for statistical significance using the Mann-Whitney U test.

Figure 2: Vaccine-induced SARS-CoV-2-specific T cells in B cell lymphoma patients after CD19 CAR T cell treatment

(A) T cells specific for the S protein of the SARS-CoV-2 were identified *ex vivo* after short-term stimulation of total PBMC using libraries of overlapping peptides covering the complete sequence of the protein. Intracellular staining of cytokines followed by flow cytometry served as a read-out assay. SARS-CoV-2-specific $CD4^+$ T cells (upper left) were defined as TNFα/CD40L (CD154)-double positive $CD3^+CD4^+$ T cells and SARS-CoV-2-specific $CD8^+$ T cells (lower left) were defined as IFNγ/TNFα-double positive $CD3^+CD8^+$ T cells. The dot plots on the left show examples of CART patient (2123-042) without any antiviral T cells prior to vaccination and easily detectable $CD4^+$ and $CD8^+$ anti-S protein T cells after the second dose of the vaccine. Background levels were typically <0.01% of all $CD4^+$ or $CD8^+$ T cells. Scatter plots indicate levels of SARS-CoV-2-specific $CD4^+$ (upper right) and $CD8^+$ T cells (lower right) prior to vaccination (11 CART patients, 7 HC) and after the 2^{nd} dose (13 CART patients, 7 HC). Gray plots on the right indicate T cell levels in 6 patients with active COVID-19. Lines indicate median levels. Differences between groups were analyzed for statistical significance using the Mann-Whitney U test. **(B)** For 5 CART patients (red), 3 B cell lymphoma (BCL) patients without prior CART treatment (yellow), and 3 HC (blue) samples were available for all three timepoints and anti-S $CD4^+$ (upper panel) and $CD8^+$ T cells (lower panel) were measured pre-vaccine, after the second dose and after the third dose. **(C)** Numbers of vaccine-induced $CD4^+$ and $CD8^+$ T cells specific for the complete sequence of the S fusion protein were compared to numbers of T cells from the same individual recognizing the N-terminal S1 protein or the N-terminal part (AA 689-895) of the S2 protein ("S+"). Dot plots on the left show exemplary data for two CART patients with $CD4^+$ (patient 2123-108) and $CD8^+$ (patient 2123-034) T cells, respectively, specific for the SARS-CoV-2 S protein. Symbols on the right show the same type of data for 3 CART patients (red) and 2 HC (blue).

Figure 1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Omicron RBD
D
CD19
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E
B cells / lymphocytes (%)
Plasma cells / lymphocytes (%)
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CART
HC vacc.
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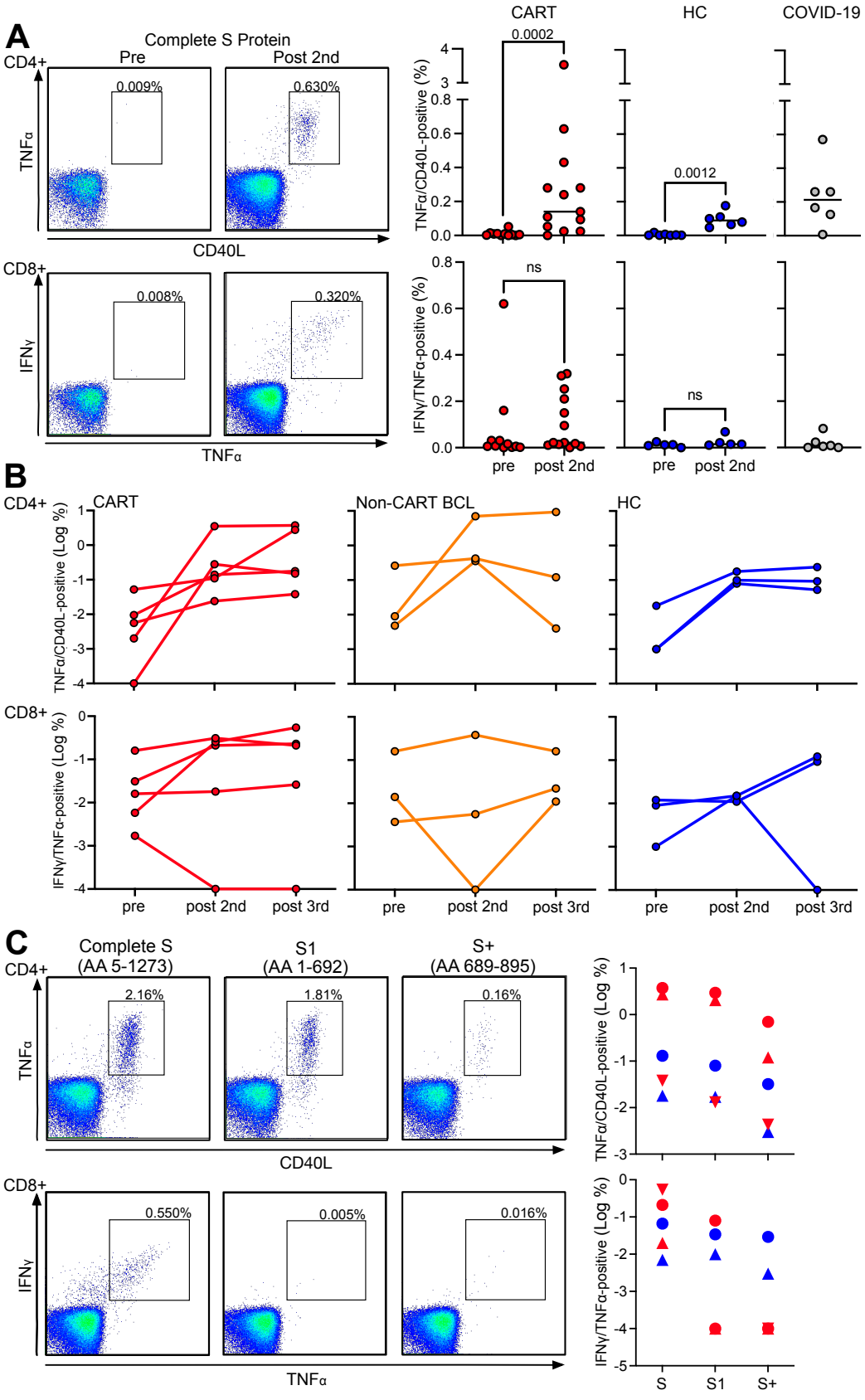
BCL
COVID

Figure 2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